Biotechnology Risk Assessment Research Grants Program—1999 Annual Report
Award No. 95-33120-1977
Title: Capability of Recombinant Insect Viruses for Environmental Persistence/Transport

James Fuxa
Louisiana State University

Specific Objectives:

1) Determine whether the recombinant viruses AcNPV.AaIT and AcNPV.JHEKK can compete with wild *Autographa californica NPV* (AcNPV.WT) for a niche in a *Trichoplusia ni* - collards microcosm.
2) Compare the attractiveness and suitability of *T. ni* killed by AcNPV.WT, AcNPV.AaIT, or AcNPV.JHEKK as food sources for the scavenging fly *Sarcophaga bullata* (Sarcophagidae).
3) Compare the survival through the gut and the defecation rate of AcNPV.WT, AcNPV.AaIT, and AcNPV.JHEKK ingested by the predator *Podisus maculiventris* (Hemiptera) or the scavenger S. *bullata*.
4) Compare the capability of AcNPV.WT, AcNPV.AaIT, and AcNPV.JHEKK to be transported over limited distances by P. *maculiventris* or S. *bullata* in a greenhouse microcosm.

Results:

1) Results of the experiment testing competition between wild-type *Autographa californica* nucleopolyhedrovirus (AcNPV or AcNPV.WT) and AcNPV expressing a scorpion toxin (AcNPV.AaIT) were presented in the September, 1998, report. The second experiment compared AcNPV.WT with AcNPV expressing insect juvenile hormone esterase (AcNPV.JHESG) in their capability to produce epizootics in *Trichoplusia ni* larvae infesting collards in a greenhouse microcosm. Larvae treated in four different ways were released into 1.8 m2 micro-plots in week 1. The four treatments included: 1) uninfected larvae (control); 2) 100% AcNPV.WT-infected larvae (WT); 3) 100% AcNPV.lHESG-infected larvae (JHESG); and 4) 1:1 ratio of AcNPV.WI-infected and AcNPV.JHESG-infected (WT+JHESG). On a weekly basis, larvae were sampled and new, uninfected larvae were added to all plots. Sampled larvae were reared until death and then subjected individually to DNA-DNA dot-blot hybridization assay for the AcNPV.WT and AcNPV.lHESG viruses to determine the proportion of insects infected with each virus in each plot. After 8 weeks or insect generations, AcNPV.WT out-competed AcNPV.JHESG for a niche in the microcosm. However, AcNPV.JHESG at 8 weeks was still causing epizootics in treatment 3, in contrast to AcNPV.AaIT in the previous experiment, which had disappeared by the third week. DNA-probe calibration curves are being completed in the laboratory of B. D. Hammock (Univ. California, Davis) to confirm probe sensitivity. After 8 weeks, soil in treatment-2 and treatment-3 plots contained similar amounts of their respective viruses. However, in treatment-4 plots, where AcNPV.JHESG and AcNPV.WT were in direct competition, the soil contained 109X more wild-type than recombinant virus. Thus, as with AcNPV.AaIT, the wild-type virus out-competed AcNPV.JHESG, which reduces the probability that the recombinant virus will persist in an agroecosystem.

2) All research in Objective 2 was completed in the report submitted in September, 1998.

3) The survival rate of wild type and recombinant nucleopolyhedroviruses through the gut and the defecation of the predatory bug, *Podisus maculiventris*, or scavenger flies *(Sarcophaga bullata)* or crickets *(Acheta domesticus)* were compared. The results for P. *maculiventris* were reported in September, 1998. The two scavengers were fed larvae of *Trichoplusia ni* killed in the fourth instar by one of the three viral variants of AcNPV. Both scavenger species voided viable NPV for at least 10 days. *Acheta domesticus* voided more viable virus than did S. *bullata,* and P. *maculiventris* voided the least virus. All three insects voided greater amounts of AcNPV.WT than AcNPV.AaIT or AcNPV.JHESG. All three insects voided sufficient amounts of all three viruses to infect >50% of the insects in a healthy host population. Thus, the recombinant viruses have the potential to be disseminated by certain predatory and scavenging insects when they are applied in the field as microbial insecticides. Furthermore, these carrier insects are all very mobile and would continuously deposit viable NPV over distances that they could travel in 10 days.

4) In four experiments in replicated greenhouse microcosms, transport of NPV by a predator and two scavengers was experimentally confirmed, and rates of transport of AcNPV.WT versus recombinant virus were compared. Experiment 1 compared transport of AcNPV.WT versus AcNPV.AaIT by *P. maculiventris*. Experiment 2 compared transport of AcNPV.WT versus AcNPV.AaIT by S. *bullata;* Experiment 3 compared transport of AcNPV.WT versus AcNPV.AalT by A. *domesticus;* and Experiment 4 compared AcNPV.WT versus AcNPVJHESG by P. *maculiventris*. Experiment 4, as well as data analyses for all experiments, are still in progress. The results of Experiments 1-3 were similar to one another: the amount of NPV transported and rates of transport were greatest in plots containing AcNPV.WT with a predator/scavenger, followed by AcNPV.WT without a predator/scavenger. Transport of AcNPV.AaIT in the presence of a predator/scavenger was a distant third, and transport of AcNPV.AaIT without a predator/scavenger was minimal. Thus, the results are confirming that a recombinant virus can be transported away from release sites by predatory and scavenging insects, which may increase the risk to possible non-target organisms. However, the amount and rate of recombinant transported is minimal compared to wild-type virus, which will be an additional factor in allowing the latter to out-compete the former for a niche in the habitat.

Plans for the Coming Year:

Objective 1: finish DNA-probe calibrations (Hammock's lab), and finish manuscript to submit to a refereed journal.

Objectives 2 and 3: Completed, except to finish manuscript to submit to a refereed journal.

Objective 4: Run the final experiment (AcNPV.WT versus AcNPV.JHESG with *Podisus),* analyze data from all four experiments, and write a refereed journal manuscript.

Publications:

Kunimi, Y., J. R. Fuxa, and B. D. Hammock. 1996. Comparison of wild type and genetically engineered nuclear polyhedrosis viruses of *Autographa californica* for mortality, virus replication and polyhedra production in *Trichoplusia ni* larvae. Entomol. Exp. Appl. 81: 251-257.

Kunimi, Y., J. R. Fuxa, and A. R. Richter. 1997. Survival times and lethal doses for wild and

recombinant *Autographa californica* nuclear polyhedrosis viruses in different instars of *Pseudoplusia includens.* Biol. Control. 9: 129-135.

Fuxa, J. R., S. A. Alaruz, A. R. Richter, L. M. Reilly, and B. D. Hammock. 1997. Capability of recombinant insect viruses for environmental persistence/transport. IN *Biotechnology Risk Assessment: Proceedings of the Biotechnology Risk Assessment Symposium, June 23-25, 1996, Ottawa, Ontario, Canada*, M. Levin, C. Grim, and J. S. Angle, Eds. Univ. Maryland Biotechnol. Inst.,College Park. Also: Http://www.nbiap.vt.edu/brarg/brasym96/brarg96.htinl

Fuxa, J. A., J. R. Fuxa, and A. R. Richter. 1998. Host-insect survival time and disintegration in relation to population density and dispersion of recombinant and wild-type nucleopolyhedroviruses. Biol. Control. 12: 143-150.

Fuxa, J. R., J.-Z. Sun, E. H. Weidner, and L. R. LaMotte. 1999. Stressors and rearing diseases of *Trichoplusia ni:* evidence of vertical transmission of NPV and CPV. J. Invertebr. Pathol. (In Press).